23208

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference BN 52 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/12610	International filing date *(day/month/year)* 12.11.2003	Priority date *(day/month/year)* 25.11.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/863, A61K39/275, A61K39/285, C12N5/10, C12N15/86		
Applicant BAVARIAN NORDIC A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 7 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.06.2004	04.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer Brouns, G Telephone No. +31 70 340-3789

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/12610

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-22 as originally filed

Claims, Numbers

1-22 received on 03.02.2005 with letter of 28.01.2005

Drawings, Sheets

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☒ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

see separate sheet

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 17,19,20

because:

☒ the said international application, or the said claims Nos. 17,19,20 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the Standard.

☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	5, 6, 10-12,19,20
	No:	Claims	1-4, 7-9, 13-18, 21,22
Inventive step (IS)	Yes:	Claims	-
	No:	Claims	1-22
Industrial applicability (IA)	Yes:	Claims	1-16,18,21,22
	No:	Claims	17,19,20 (?)

2. Citations and explanations

see separate sheet

The present application discloses the use of a vaccinia vector comprising two expression cassettes with promoters of the cowpox A-type inclusion protein gene (ATI) in tandem inserted into the same insertion site. The resulting vector is stable, allows expression of two different genes at similar levels and is suitable for use in the preparation of a vaccine.

Re Item I
Basis of the report

Amended claims 1-22 filed with the letter of 28.01.2005 fulfil the requirements of Article 34(2)(b) PCT, since a basis for said claims may be found in the application as filed. Amended pages 1 and 2 and the figure filed with the letter of 28.01.2005 are not allowable, since they go beyond the disclosure of the international application as filed.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

Claims 17, 19 and 21 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1) Reference is made to the following documents:

D1: HOWLEY P M ET AL: "A vaccinia virus transfer vector using a GUS reporter gene inserted into the I4L locus" GENE, ELSEVIER BIOMEDICAL PRESS. AMSTERDAM, NL, vol. 172, no. 2, 26 June 1996 (1996-06-26), pages 233-237

D2: HÄNGGI M ET AL: "Conserved TAAT motif in Vaccinia virus late promoters

overlapping TATA box and site of transcription initiation", EMBO JOURNAL, vol. 5, no. 5, 1986, pages 1071-1076

D3: WO9702355 (SUTTER GERD; ERFLE VOLKER (DE); OHLMANN MARION (DE); GSF FORSCHUNGZE) 23 January 1997 (1997-01-23)

D4: BOYLE D B ET AL: "Construction of recombinant Fowlpox viruses as vectors for poultry vaccines" VIRUS RESEARCH, AMSTERDAM, NL, vol. 10, no. 4, June 1998 (1998-06), pages 343-356

NOVELTY (Article 33(2) PCT)

2.1) D1 discloses a poxviral vector, vaccinia virus Copenhagen, comprising up to four p7.5 promoters regulating expression of different coding sequences, amongst which the measles virus nucleoprotein as antigen (D1, figure 2; page 236, right-hand column, paragraph 2; page 237, last paragraph). Said viral vectors have been used for infection of BHK cells and transcripts from the p7.5 promoters have been detected.

The vector of D1 has been shown to recombine and is therefore unstable, whereas it seems to be an object of the present invention to provide **stable** recombinant poxviral vectors.
However, since the vaccinia virus late promoter p7.5 (D2, table I) has a homology of 65% over 29 nucleotides when compared to the sequence of SEQ ID NO:1, and comprises nucleotides 25-29 of said sequence, it may be considered as an 'cowpox ATI promoter derivative'. There are no technical features disclosed in the application that allow the skilled person to distinguish between the poxviral vector of D1 and the present application, therefore the subject-matter of claims 1-4, 7-9, 13-18, 21 and 22 lacks novelty (Article 33(2) PCT).

2.2) D3 (abstract; fig. 5) and D4 (fig. 2) disclose poxviral vectors comprising two expression cassettes in the same insertion site, comprising each a promoter that may be considered a 'derivative of a cowpox ATI promoter': p7.5 as discussed above, and p11 which shares 68% homology with a subsequence of the cowpox ATI promoter defined by SEQ ID NO:1 (nucleotides 14-29) and comprises nucleotides 25-29 of SEQ ID NO:1.
D3 and D4 therefore anticipate the subject-matter of claims 1-3, 7-9, 13-18, 21 and 22.

2.3) The subject-matter of claims 5, 6, 10-12, 19 and 20 is not disclosed in the prior art,

therefore said claims are novel.

INVENTIVE STEP (Article 33(3) PCT)

3) It is an object of the present invention to provide stable poxviral vectors comprising at least two expression cassettes, each comprising the cowpox ATI promoter or a derivative thereof. As indicated above, the prior art discloses an unstable poxviral vector comprising at least two expression cassettes, each comprising a promoter that may be considered a cowpox ATI promoter derivative, for the development of a polyvalent vector.
Not all claimed cowpox ATI promoters are therefore suitable to practise the invention and it is not indicated in the application what essential technical feature of the cowpox ATI promoter is required to solve the stated problem of 'providing a stable poxvirus'.

A practical example is provided in which two expression cassettes, each comprising a cowpox ATI promoter with the sequence defined by SEQ ID NO:1, has been shown to provide a stable poxviral vector (description, lines 22, 23).

Since no effect is demonstrated in the application for the use of the claimed **derivatives** of the cowpox ATI promoter defined by SEQ ID NO:1, no inventive step may be acknowledged for the subject-matter of claim 1-22 (Article 33(3) PCT).

INDUSTRIAL APPLICABILITY (Article 33(4) PCT)

4) For the assessment of the present claims 17, 19 and 21 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

***

JC20 Rec'd PCT/PTO 04 MAY 2005

International Application
PCT/EP2003/012610
Amended claims enclosed to
the response to the Written Opinion

Claims:

1. Recombinant poxvirus comprising in the viral genome at least two expression cassettes, each comprising the cowpox ATI promoter or a derivative thereof or a subsequence of the ATI promoter or the derivative thereof and a coding sequence, wherein the expression of the coding sequence is regulated by said promoter, derivative or subsequence and wherein the derivative of the cowpox ATI promoter is a sequence that has a homology of at least 60% when compared to the sequence of SEQ ID.: No. 1 and/or a sequence in which not more than 6 nucleotides are substituted, deleted and/or inserted in the sequence of SEQ ID.: No.1, wherein the subsequence of the ATI promoter has a length of at least 10 nucleotides of the sequence of SEQ ID.: No. 1 and wherein the promoter, derivative or subsequence has the biological activity of being active as a promoter.

2. Recombinant poxvirus according to claim 1, wherein the promoter, derivative or subsequence has the biological activity of being active as a Vaccinia virus late promoter.

3. Recombinant poxvirus according to anyone of claims 1 to 2, wherein the promoter, derivative or subsequence comprises nucleotides 25 to 29 or 22 to 29 of SEQ ID.: No.1.

4. Recombinant poxvirus according to anyone of claims 1 to 3, wherein the promoters, derivatives or subsequences in the recombinant poxvirus are the same.

5. Recombinant poxvirus according to anyone of claims 1 to 4, wherein at least two expression cassettes are inserted into the same insertion site in the poxvirus genome.

6. Recombinant poxvirus according to anyone of claims 1 to 5, wherein the promoter in at least one of the expression cassettes has the sequence of SEQ ID: No. 1

7. Recombinant poxvirus according to anyone of claims 1 to 6, wherein the promoter in at least one of the expression cassettes is a derivative of the ATI promoter or a subsequence of the ATI promoter or a derivative thereof.

8. Recombinant poxvirus according to anyone of claims 1 to 7, wherein the poxvirus is selected from the group consisting of orthopoxviruses and avipoxviruses.

9. Recombinant poxvirus according to claim 8, wherein the orthopoxvirus is a vaccinia virus and wherein the avipoxvirus is selected from canarypoxvirus and fowlpoxvirus.

10. Recombinant poxvirus according to claim 9, wherein the vaccinia virus is modified vaccinia virus strain Ankara (MVA), in particular MVA-BN and MVA 575, deposited under numbers V00083008 and V00120707, respectively, at the European Collection of Animal Cell Cultures (ECACC).

11. Recombinant poxvirus according to claim 10, wherein at least one of the expression cassettes is inserted in a naturally occurring deletion site of the MVA genome with respect to the genome of the vaccinia virus strain Copenhagen.

12. Recombinant poxvirus according to anyone of claims 1 to 11, wherein at least one of the expression cassettes is inserted in an intergenic region of the poxvirus genome.

13. Recombinant poxvirus according to anyone of claims 1 to 12, wherein at least one of the coding sequences codes for least one antigen, antigenic epitope, and/or a therapeutic compound.

14. Recombinant poxvirus according to anyone of claims 1 to 13 as vaccine or medicament.

15. Vaccine or pharmaceutical composition comprising a recombinant poxvirus according to anyone of claims 1 to 13.

16. Use of the recombinant poxvirus according to anyone of claims 1 to 13 for the preparation of a vaccine or medicament.

17. Method for introducing coding sequences into target cells comprising the infection of the target cells with the virus according to anyone of claims 1 to 13.

18. Method for producing a peptide, protein and/or virus comprising

a) infection of a host cell with the recombinant poxvirus according to anyone of claims 1 to 13,

b) cultivation of the infected host cell under suitable conditions, and

c) isolation and/or enrichment of the peptide and/or protein and/or viruses produced by said host cell.

19. Method for affecting, preferably inducing an immunological response in a living animal body including a human comprising administering the virus according to anyone of the claims 1 to 13 or the composition or vaccine according to claim 15 to the animal or human to be treated.

20. Method according to claim 19 comprising the administration of at least 10^2 $TCID_{50}$ (tissue culture infectious dose) of the virus.

21. A cell containing the virus according to any of claims 1 to 13.

22. A method for the production of a recombinant virus according to anyone of claims 1 to 13 comprising the step of inserting at least two expression cassettes into the genome of a poxvirus.

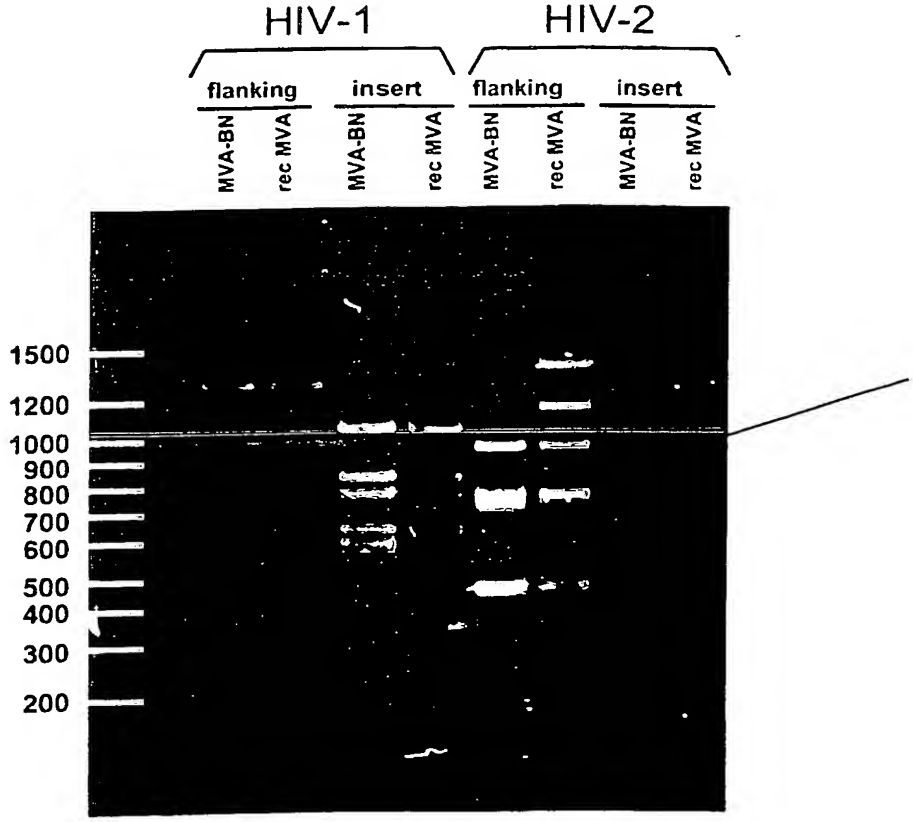
Proof of stability for two ATI promoters inserted at two different intergenic regions

This investigation was done in order to prove that a recombinant virus based on the MVA-BN vector technology is stable although two cowpox ATI promoters are inserted at two different intergenic regions (IGRs) of MVA-BN (see WO02/42480). Therefore we were generating a virus carrying two HIV genes (termed "HIV1" and "HIV2") in two different IGRs, namely IGR 44/45 at position 37,000 and IGR I4L/I5L at position 56,700. Both genes were driven by the same ATI promoter sequence. The virus was plaque purified and tested after more than 20 passages..

To demonstrate the stability of our construct we were amplifying the regions next to the two insertions (~5kb in length) and the regions spanning the inserted genes (~5.5kb in length). The reactions were carried out with the recombinant virus (recMVA) and the wild type MVA-BN as control. The obtained eight PCR fragments were digested with restriction enzymes to get a specific restriction pattern. The fragments were analyzed on a 1% agarose gel. With the help of the restriction pattern it should be possible to make occurring instabilities visible, as deletions or insertions would change the restriction pattern. The size of the expected fragments for the wild type virus (MVA-BN) and the recombinant MVA-BN (recMVA) are shown below the enclosed figure.

Our results show, that the two PCRs of the surrounding area show the same pattern for the recMVA as for the wild type MVA-BN indicating that these regions are stable although they have genes inserted next to it. The two PCRs spanning the regions with the insertions show small differences between MVA-BN and the recMVA, which account for the inserted genes. For HIV-1 of the recMVA-BN there is an additional 628 bp band next to the 637bp band, the latter being visible in both, MVA-BN and recMVA. Moreover, a 128 bp band is missing in the recMVA-BN. For HIV-2 MVA-BN has an additional 877bp band and recMVA-BN has two additional bands, one at 565 and one at 354 which are next to 355, the latter being visible in both, recMVA and MVA-

BN. Thus, the pattern of all four PCRs for wild type and recombinant virus match exactly with the predicted fragment sizes leading to the conclusion that our recombinant virus is stable. The experiments have not given any hint that the recombinant MVA comprising two ATI promoters in the genome might be instable or show a tendency towards homologous recombination between the ATI promoter sequences.



HIV-1

MVA-BN	1047, 832,765, 637, 591, 565, 335, 191, 146, **128**, 2
recMVA-BN	1047, 832, 765, 637, **628**, 591, 565, 335, 191, 146, 2

HIV-2

MVA-BN	980, **877**, 565, 547, 477, 438, 355, 212, 168, 165, 129, 94
recMVA-BN	980, 565, **564**, 547, 477, 438, 355, **354**, 212, 182, 168, 129, 103, 102, 94, 81, 56, 55, 46, 35, 28